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Mini review

Integrins and chondrocyte–matrix interactions in articular cartilage

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ABSTRACT

The integrin family of cell adhesion receptors plays a major role in mediating interactions between cells and the extracellular matrix. Normal adult articular chondrocytes express α1β1, α3β1, α5β1, α10β1, αVβ1, αVβ3, and αVβ5 integrins, while chondrocytes from osteoarthritic tissue also express α2β1, α4β1, α6β1. These integrins bind a host of cartilage extracellular matrix (ECM) proteins, most notably fibronectin and collagen types II and VI, which provide signals that regulate cell proliferation, survival, differentiation, and matrix remodeling. By initiating signals in response to mechanical forces, chondrocyte integrins also serve as mechanotransducers. When the cartilage matrix is damaged in osteoarthritis, fragments of fibronectin are generated that signal through the α5β1 integrin to activate a pro-inflammatory and pro-catabolic response which, if left unchecked, could contribute to progressive matrix degradation. The cell signaling pathways activated in response to excessive mechanical signals and to fibronectin fragments are being unraveled and may represent useful therapeutic targets for slowing or stopping progressive matrix destruction in arthritis.

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1. Introduction

1.1. Introduction to integrins

Integrins are heterodimeric transmembrane proteins consisting of α and β subunits with large extracellular domains that cooperate in binding to matrix ligands, and short cytoplasmic domains, that lack intrinsic kinase activity but which interact with proteins that initiate kinase-mediated intracellular signaling (reviewed in Giancotti and Ruoslahti (1999), Hynes (2002), Legate et al. (2009)). The integrin cytoplasmic tails also bind to and help organize cytoskeletal proteins including talin, paxillin, vinculin, tensin and actin (Wolfenson et al., 2013). Thus, integrins function to "integrate" the extracellular matrix (ECM) with cytoskeletal structures and signaling components. Signals mediated by integrins also cross talk with signals generated by soluble factors, including growth factors and cytokines (Danen and Yamada, 2001; Legate et al., 2009; Schwartz and Ginsberg, 2002). In response to extracellular cues, integrins can therefore regulate many important cellular functions including cell proliferation, differentiation, survival, and migration as well as tissue morphogenesis and remodeling.

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There are 24 different integrin heterodimers formed by the combination of 8 types of β subunits and 18 types of α subunits (Hynes, 2002). In general, the β1 and αV subfamilies mediate cell–matrix interactions and will be the focus of this review while the β2 subfamily mediates cell-cell adhesions and are mainly present on leukocytes. Each integrin heterodimer can recognize and bind one or more different ECM proteins and various ECM proteins can bind to one or more different integrin heterodimers. For example, the matrix protein fibronectin has binding sites for α3β1, α4β1, α5β1, and αVβ1 integrins while the collagen binding integrins include α1β1, α2β1, α10β1, and α11β1. The integrin repertoire expressed by a given cell is influenced by the composition of the surrounding ECM as well as by signals generated by soluble factors, such as growth factors, that regulate integrin expression.

Integrin binding of ECM proteins results in stimulation of signaling networks that include multiple tyrosine and serine kinases as well as adapter proteins ("outside-in" signaling) (Hynes, 2002; Legate et al., 2009). In many cell types, focal adhesion kinase (FAK) is a key upstream mediator of integrin signaling and serves as a major hub for integrating signals generated by integrins and growth factors (Legate et al., 2009). Many of the signaling intermediates activated by integrin ligation converge on the mitogen activated protein (MAP) kinase family which includes ERK, JNK, and p38 resulting in downstream regulation of gene transcription (Giancotti and Ruoslahti, 1999; Legate et al., 2009). Depending on the context of activation, the MAP kinase family can also mediate signals generated by anabolic factors, such as growth factors, as well as catabolic factors, such as cytokines, and by mechanical stimuli which also activate integrin signaling. In this way, integrin signals work in concert with signals generated by growth factors, cytokines and mechanical forces making them very important mediators of signaling events relevant to tissues such as articular cartilage.

1.2. Chondrocyte integrin expression in normal and arthritic cartilage

Normal adult articular chondrocytes primarily express α1β1, α3β1, α5β1, α10β1, αVβ1, αVβ3, and αVβ5 integrins (Camper et al., 1998; Loeser et al., 1995, 2000; Ostergaard et al., 1998; Salter et al., 1992; Woods et al., 1994). In OA cartilage, there appears to be an increase in levels of α1β1 and α3β1 (Loeser et al., 1995) along with the appearance of α2β1, α4β1 and perhaps some α6β1 not detected in normal cartilage (Lapadula et al., 1997; Ostergaard et al., 1998). The mechanism responsible for a change in integrin expression in OA tissue has not been determined but could relate to the effects of growth factors and cytokines that stimulate integrin expression and are present in OA issue, as well as feedback regulation from changes in the ECM and promotion of chondrocyte hypertrophy resulting in expression of integrins seen on hypertrophic chondrocytes (Arner and Tortorella, 1995; Hausler et al., 2002; Jobanputra et al., 1996; Loeser, 1997).

2. Integrin–matrix interactions in cartilage

2.1. Integrin-mediated binding of chondrocytes to extracellular matrix proteins

A major function of integrins is to mediate cell adhesion to the ECM and a number of studies have examined integrin-mediated adhesion of chondrocytes to cartilage matrix proteins. The major chondrocyte integrins and the ECM proteins to which they bind are shown in Table 1. The α5β1 and αVβ3 integrins recognize and bind to the Arg-Gly-Asp (RGD) sequence present in many ECM proteins and short synthetic RGD peptides can be used to inhibit this binding in adhesion assays. This technique was shown in an early study to inhibit adhesion of adult chondrocytes to fibronectin, osteopontin, bone sialoprotein, and vitronectin, which contain RGD sequences, and to matrix Gla protein (MGP) which does not (Loeser, 1993).

Table 1
Chondrocyte integrins and their ligands.

Integrin	Extracellular matrix proteins
α1β1	Collagen types VI and II, matrilin-1
α2β1 (OA chondrocytes)	Collagen type II and VI, chondroadherin
α3β1	Fibronectin
α4β1 (OA chondrocytes)	Fibronectin
α5β1	Fibronectin
α6β1 (OA chondrocytes)	Laminin
α10	Collagen type II
αvβ1	Fibronectin, vitronectin, osteopontin
αvβ3	COMP, fibronectin, vitronectin, osteopontin
αvβ5	Fibronectin, vitronectin, osteopontin

The binding of chondrocytes to MGP was likely indirect and mediated by fibronectin which binds to MGP (Cancela et al., 1994), demonstrating the complex interactions between the ECM and integrins. A similar interaction with α5β1 has been observed with fibronectin and connective tissue growth factor (Hoshijima et al., 2006). RGD-dependent binding of chondrocytes has also been observed with thrombospondin 1 (Miller and McDevitt, 1995) and with cartilage oligomeric matrix protein (COMP), the latter of which is through the αVβ3 integrin (Chen et al., 2005). Adhesion of chondrocytes to the cut surface of articular cartilage was inhibited using antibodies to β1, α5β1, and αVβ3 under conditions of flow in order to study chondrocyte–matrix interactions that may be important in cell-based repair (Kurtis et al., 2003).

The primary type II collagen binding integrin expressed by normal adult chondrocytes is α10β1 (Camper et al., 1998) while α1β1 can bind type II collagen but may prefer type VI collagen (Loeser et al., 2000). Unlike cells from normal cartilage, OA chondrocytes express α2β1 (Lapadula et al., 1997; Ostergaard et al., 1998) which can bind type II collagen (Loeser et al., 2000) as well as chondroadherin (Haglund et al., 2011). Binding of chondrocytes to cartilage matrix protein (matrilin-1) can be mediated by α1β1 through an interaction with type II collagen (Makihira et al., 1999). Complex interactions with collagen, and perhaps other ECM proteins, also appear to mediate binding of chondrocytes to laminin via the α6β1 integrin (Durr et al., 1996).

Integrin blocking antibodies have been used to examine functions mediated by integrin–ECM interactions. Studies using isolated chick sternal chondrocytes found that blocking α1, α2, or β1 integrins reduced survival and hypertrophic differentiation (Hirsch et al., 1997) while studies in mouse limb organ culture using antibodies to α5β1 found that this integrin also plays a role in chondrocyte differentiation and joint formation (Garciadiego-Cazares et al., 2004). Similar ex vivo experiments used injections into the upper limbs of mouse embryos of antibodies to α5β1 or RGDS peptides to support a role for the α5β1 integrin in endochondral ossification (Inoue et al., 2014).

In adult articular chondrocytes, inhibition of the α5 integrin subunit reduced cell survival in serum-free culture in alginate and inhibited the ability of IGF-1, but not serum, to prevent cell death (Pulai et al., 2002). In monolayer cultures, treatment with α5β1 or αVβ5 antibodies inhibited the de-differentiation that occurs over time as the cells attach and spread. Together, these in vitro studies provided evidence that, like other cell types, integrin-mediated interactions with the ECM are important for cell survival and differentiation.

2.2. Effects of chondrocyte integrin deficiency in mice

A few studies have evaluated the effect of integrin deficiency on cartilage in knock-out mice in order to study integrin function in vivo. Mice homozygous for a null mutation in the β1 integrin subunit die at a very early embryonic stage (Sheppard, 2000) and so floxed β1 integrin mice were crossed with the Col2a1-cre mice to delete β1 integrins in chondrocytes. These mice were found to develop a chondrodysplasia